A Research Note

Spoilage of Soft Drinks Caused by Bacterial Flocculation1

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ABSTRACT

Fruit-flavored soft drinks (pH 3.0) spoiled due to flocculation caused by strains of Acetobacter spp. The floe consisted of bacterial cells attached to cellulose microfibrils. Floe production was inhibited at 4°C; it was not prevented by addition of 200 ppm benzoate, 200 ppm sorbate or 100 ppm sulfur dioxide.

Lactic and acetic acid bacteria are the groups usually involved in bacterial spoilage of high-acid food products (pH <4.0). Spoilage caused by those bacteria is mainly due to production of off-flavors, but may also result from visible changes in the product. Certain gram-negative bacteria, and among them species of the genus Acetobacter, produce exocellular cellulose microfibrils in broth cultures and this may lead to flocculation (2,6). This paper describes a spoilage problem in soft drinks (pH~3.0) caused by bacterial flocculation.

MATERIALS AND METHODS

Soft drink samples

Samples of orange- and apple-flavored soft drinks were received from one manufacturer. They had the following characteristics: pH 2.9-3.0, Brix 8-10°C, and titratable acidity of ca. 90 milliequivalent acid per liter. They were pasteurized by heating for 3 min at 90°C, cooled, aseptically filled into pre-sterilized polyethylene pouches, and marketed at ambient temperature.

Identification of spoilage organisms

Dilutions in sterile aqueous sucrose solution (0.3 mol/l) were prepared from samples showing flocculation and plated in citric acid (CA) agar (pH 5.5) (5). The plates were incubated aerobically at 30°C for 2-5 d. Isolated colonies were purified by streaking onto plates of CA agar. Pure cultures were used to inoculate filter-sterilized samples of orange-flavored drink, which were then incubated at 30°C. Flocculation was followed visually; cultures able to produce flocculation within 5 d were taken for identification. Acetic acid bacteria were tested, to allow identification, according to the criteria described by Carr and Passmore (1).

Floe analysis

The floe was analyzed microscopically. Cellulose was identified by birefringence under polarized light and formation of a blue adsorption compound with iodine solution after hydrolysis by H2SO4 (4).

Chemicals

Sodium benzoate (100 and 200 ppm), potassium sorbate (100 and 200 ppm), sulfur dioxide (100 ppm) and ascorbic acid (1,000 ppm) were added to CA broth, and to filter-sterilized drink, and their influence upon floe formation by the selected bacterial cultures was followed at 30°C.

Storage

To test the effect of storage temperatures, samples of filter-sterilized orange-flavored drink were inoculated with floc-producing cultures (ca. 103 organisms/ml), and then incubated up to 2 weeks at 4, 10, 20 and 30°C.

RESULTS AND DISCUSSION

In certain batches of orange- and apple-flavored soft drinks, spoilage by flocculation was observed 2-3 d after they had been processed. Spoiled products had pH values in the range of 2.7 to 2.9. Bacteria were isolated from the flocculated soft drink samples which were able to cause flocculation in a filter-sterilized drink (pH 3.0) or in CA broth (pH 5.5). They had the following characteristics: short, coccoidal gram-negative rods; motile; occurring singly, in pairs or short chains; catalase-positive; oxidase-negative; and exhibiting abundant growth under aerobic conditions. On the basis of those characteristics plus their acid tolerance they were classified as acetic acid bacteria. They produced acid and a brown pigment in glucose medium, and acetic acid from ethyl alcohol. Microscopic examination of the floe produced by these bacteria revealed a network of microfibrils, further identified as cellulose, to which the cells were attached (Fig. 1-3). With the exception of brown pigment production, the floc-producing isolates fit the genus Acetobacter and will be referred to hereinafter as Acetobacter spp. They can be considered as atypical strains of either Acetobacter

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Floc formation in an orange-flavored drink inoculated with Acetobacter sp. 1. Exocellular fibrils, consisting of cellulose, are produced by the growing culture, × 3,000. 2. Cellulose microfibrils, × 1,200. 3. Bacterial cells attach to the fibrils creating a floc, × 3,000.

zyminum or Acetobacter aceti. Atypical strains of A. aceti have been described which produce cellulose as well as a brown pigment from glucose (1).

In filter-sterilized orange-flavored drink, inoculated with Acetobacter spp., flocculation occurred after 2 d at 30 or 20°C, 5 d at 10°C, but did not occur within 2 weeks of storage at 4°C. Ferguson and Powrie (3) found that ascorbic acid inhibited growth of acetic acid bacteria in apple juice. The possibility of inhibiting floc formation by addition of antimicrobial preservatives or by creating conditions of low redox-potential was considered. The spoilage process was not inhibited and floc production by Acetobacter spp. occurred after 2-3 d at 30°C when the following chemical preservatives were added at concentrations normally used in the manufacture of the soft drinks: sodium benzoate (200 ppm), potassium sorbate (200 ppm), benzoate + sorbate (100 + 100 ppm), sulfur dioxide (100 ppm) or ascorbic acid (1000 ppm).

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